SO-CALLED PARTHENOGENESIS IN THE WHITE MOUSE.

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In atretic follicles of ovaries in a number of different mammals the oöcytes go through a process which somewhat resembles maturation. Various stages of mitosis are seen and frequently a first polar body is present. In some cases the oöcyte is found divided up in a number of small parts some of which contain nuclei. This process has been described as a beginning of parthenogenetic cleavage and also as degenerative fragmentation. Bonnet ('00) gives a review of the work done up to that time and after considering all the evidence decides that the mitotic figures seen in such egg-cells are not those of parthenogenetic cleavage but are rather those of more or less abnormal maturation stages. Newman ('13) reviews briefly the work done since Bonnet's paper and presents the results of his studies on the armadillo in support of the view that "a limited amount of parthenogenetic cleavage occurs but that development proceeds no farther than two or three cell divisions." Van der Stricht ('01), who worked on the bat claims that in that form is found a beginning of true parthenogenesis. Rubaschkin ('07), who studied the guinea-pig, and Athias ('09), for the dormouse, state that the phenomena are to be interpreted as degenerative fragmentation which at the most merely resembles parthenogenesis.

In material which I have been preparing for a study of oögenesis in the white mouse I found that in the ovaries of young mice approaching sexual maturity there is a very extensive degeneration of follicles, most marked between the ages of twenty-five and forty days. As the work on this problem has all apparently been done on the ovaries of fully or young adult mammals, it was thought worth while to use the material in these immature ovaries[1] for a study of this so-called parthenogenesis.

[1] The material used for this work consists of ovaries of white mice varying in age from twenty to ninety days. These ovaries were fixed in Carnoy's fixer

In the white mouse, it is certain that the changes taking place in the oöcytes are in some way correlated with the atresia of the follicles, for in follicles which have not begun to degenerate the egg-cells are normal in appearance and the nuclei are normal resting nuclei. But in follicles overtaken by atresia the cytoplasm of the oöcytes is found to stain more deeply with acid stains, fat granules are found in large numbers, and the nuclei are in various stages of mitosis. The degeneration of the follicle in some way stimulates the oöcyte so that it passes through more or less abnormal maturation stages.

The early prophase is probably passed through very rapidly as no stages were seen of nuclei between the resting stage and the equatorial plate stage. Lams and Doorme ('08) did not observe the stages between the resting nucleus and the first polar spindle in the normal maturation of the egg of the white mouse. Kirk-

("6:3:1"), Hermann's, and Flemming's fluids. Heidenhain's iron hematoxylin, Jenner's blood stain, and Flemming's triple stain were used. Sections were stained over night in Jenner's stain, diluted with three parts of water; this stain was used after fixation in Carnoy's fluid and gave excellent results for some purposes. The cytoplasm of degenerating egg-cells was stained a much deeper pink or red than that of normal egg-cells. The nuclei of follicle cells and phagocytic cells were stained a deep blue. The stain will fade after a time, however.

A modification of theshorter method of Flemming's triplestain was used. Sections of material fixed for two to four hours in Flemming's or Hermann's fluid were bleached in a dilute solution of hydrogen peroxid and after rinsing were placed in a four per cent. solution of ferric alum for four to twelve hours. The sections were then rinsed in distilled water, dipped in the safranin solution a second or two, rinsed again in distilled water, and placed in the gentian violet solution for two to ten or fifteen minutes. Then after rinsing in distilled water the sections were stained in the orange G for ten to sixty seconds. After dehydrating rapidly in absolute alcohol the sections were differentiated in clove oil under the control of the microscope. The clove oil was removed by toluene or xylene and the sections mounted in balsam. By this method the cytoplasm of the oöcytes was stained a yellow-brown, the chromosomes were stained a violet, and the spindle fibers a dark violet—almost purple. The method is rather capricious but when successful, the spindle fibers stand out very distinctly against the yellow-brown cytoplasm of the egg-cell. The method was used principally to bring out the spindle fibers, as the chromosomes are not stained so distinctly or sharply as by the iron hematoxylin method.

The solutions used are as follows:

1. Safranin, saturated solution in absolute alcohol, 1 part.
   Safranin, saturated solution in distilled water, 1 part.
2. Gentian violet, 1 per cent. solution in distilled water.
3. Orange G, 2 per cent. solution in distilled water.

ham ('08) describes very briefly a few stages of the prophase of the first maturation division in this same form.

Descriptions of the first and second polar spindles in normally maturing oöcytes do not agree. Sobotta ('07) says: "Wenn auch namentlich die Breite individuell etwas wechselt, so beträgt Länge wie Breite des ersten Richtungsspindel der Maus doch stets das Doppelte der Masse des zweiten Richtungsspindel, die Breite ist in der Regel mehr als doppelt so gross." Lams and Doorme, on the other hand, find that the first and second polar spindles are of exactly the same length and diameter. They state: "D'une façon certaine, un ovule à second fuseau ne se distingue d'un ovule à premier fuseau que par la présence, dans le premier cas, du premier globule polaire." Kirkham agrees with Sobotta in saying that the first polar spindle is larger than the second and also in describing the chromosomes as differing in size and shape in the two spindles.

In regard to oöcytes in atretic follicles, Athias says of the spindle, first or second: "Sa forme et ses dimensions sont variables, mais il n'y a pas des caractères qui soient propre au premier ou au second fuseau; la présence concomitante d'un premier globule polaire est, d'après ce que j'ai pu constater dans mes préparations, le seul critérium pour affirmer si l'on est en présence d'un second fuseau de direction." In my own preparations the lengths and diameters of a number of first and second polar spindles in atretic oöcytes were measured. For the first polar spindles, the average length was found to be 24.7 μ, the diameter 12.8 μ, while for the second, the average length was 25.9 μ and the diameter 8.7 μ. Allowing for error in measuring, the spindles are seen to be of about the same length, while the second polar spindles are about two thirds the diameter of the first.

In degenerating oöcytes of the white mouse the first polar spindles are found to be of two general forms: slender, with the achromatic fibers meeting at a point at each end, and thick, "barrel-shaped," with broadly rounded ends. These two kinds are met with in about equal numbers. In both, the chromosomes are arranged with their long axes parallel to that of the spindle. The chromosomes are not arranged around the periphery of the

spindle, but are scattered in the plane through the middle of the spindle, at right angles to its length. The chromosomes of the first polar spindle are larger than those of the second and are long with a marked thickening or swelling at the middle. This thickening is at one side of the chromosomes and it is at this point that division takes place. The division is apparently transverse. This account agrees with those of Sobotta and Kirkham for the normal first maturation spindle.

The chromsomes are grouped so closely together and overlap to such an extent that it is difficult to determine their number with accuracy. They apparently vary in number from twelve to twenty-four, the larger numbers being due to a precocious division of some, while others are still undivided.

Descriptions of the appearance of the spindle itself differ. Sobotta, Kirkham, and Lams and Doorme, state that polar radiations or asters are not present in first polar spindles of normally maturing oöcytes, and Athias agrees with them in the case of degenerating egg-cells. Rubaschkin, however, describes in atretic oöcytes of the guinea-pig polar radiations arising from a clear area or centrosphere. Kirkham describes centrosomes consisting of several minute granules at the poles of the spindles. Sobotta states that there are no centrosomes in the first polar spindles of normally maturing oöcytes, and Athias finds none in degenerating egg-cells. In my own preparations radiating fibers are to be seen at the poles of a few first polar spindles; these, however, are not to be considered true asters, but spindle fibers which have broken away from the spindle. This will be discussed more in detail further on. No centrosomes are found in any of these degenerating oöcytes. The spindle fibers are not divided into central spindle fibers and mantle fibers; the fibers with chromosomes attached are intermingled with those which are not connected with chromosomes. The two kinds do not differ in appearance or staining reaction. The spindle itself usually lies at right angles to the radius of the oöcyte, until it swings around to a radial position for the formation of the polar body. (See Figs. 1, 2 and 5.)

The stages in the formation of the first polar body must follow one another rapidly for only a few of these stages were

observed. Some of the chromosomes divide earlier than others and consequently the metaphase is not so distinctly marked as in some forms. A few instances of a telophase were seen, in some of which both groups of daughter-chromosomes are in the oöcyte, with no indication as yet of a division of the cytoplasm to form the polar body, while in the others the constricting off of the polar body may be plainly seen. After the first polar body is formed the chromosomes remaining in the oöcyte do not form a resting nucleus but at once enter the second polar spindle.

The second polar spindle, as stated above, is of about the same length as the first, while only two thirds or three fourths as much in diameter. The chromosomes as seen in the equatorial plate stage are short and rod-like and straight or slightly curved. They are not as long or as much curved as Kirkham describes in the second polar spindles of normally maturing oöcytes. The chromosomes are arranged with their long axes parallel to that of the spindle itself and as in the first polar spindle are scattered in a plane at right angles to the length of the spindle. This differs from Kirkham's account of the normal second polar spindle, in which he states that in general the chromosomes lie with their long axes across the spindle. In the spindles of these degenerating egg-cells some of the chromosomes are constricted across the middle in preparation for division, having the appearance of elongated dumb-bells; others have not started to divide and have the typical rod-like form. Others still have already divided and are short and thick, their length only slightly exceeding their diameter. (See Figs. 3 and 4.) This division as well as the first is apparently transverse in the mouse. The chromosomes are crowded together, as in the first polar spindle, making an accurate count difficult; there are from twelve to thirty, owing to the fact that some divide sooner than others.

Descriptions of the appearance of the second polar spindle are as conflicting as those of the first. Sobotta states that in normally maturing oöcytes there are neither centrosomes nor polar radiations. Lams and Doorme describe centrosomes but say that polar radiations are absent; Kirkham states that both centrosomes and polar radiations are present in some cases. In second polar spindles of degenerating oöcytes Athias states

that neither centrosomes nor polar radiations are present. In my own preparations radiating fibers are to be seen at the poles of a number of second polar spindles. As in the case of the first polar spindles, however, these are not to be considered true asters, but spindle fibers which have broken away from the spindle, and have assumed a radial position at the ends of the spindles. This will be discussed more in detail later. In general, centrosomes are absent in second polar spindles of atretic oöcytes, but are present in some cases. When they are seen, they consist of a few minute granules which stain deeply and are either in a compact group at the poles of the spindle or are somewhat spread out forming a sort of cap for the spindle. (See Figs. 3, 4 and 6.)

The first polar body is almost always present with the secondary oöcyte in atretic follicles, although in a few instances the spindle has all the characteristics of a second polar spindle while the polar body is not to be seen. In such cases it is possible that the polar body has already degenerated and been absorbed, or as Kirkham suggests for normal oöcytes, it may have been "forced through the zona (pellucida) by the contraction of the latter under the influence of changing osmotic conditions" during fixation. In nearly every instance, however, the polar body is present, lying within the zona pellucida, and somewhat flattened between the oöcyte and the zona. The smaller dimension of the polar body is one half or two thirds of the larger, while the larger diameter itself is a fifth to a third that of the oöcyte. In a few cases the polar body contains a spindle more or less deranged and abnormal (see Fig. 3) but usually the chromosomes are scattered through it irregularly. They may be grouped in a few large irregular masses of chromatin or there may be a number of smaller chromosomes of abnormal size and shape. In a few cases a resting nucleus may be seen in the polar body (Fig. 10). The second polar spindle is usually found in the oöcyte near the polar body, at right angles to the radius of the egg-cell. Rarely it may be seen in the other side of the oöcyte, and a few spindles have been seen in a radial position. A few instances were observed where the oöcyte contained two spindles; this is probably due to the fact that the egg-cell had two nuclei to start with.

Up to this point oöcytes in follicles undergoing follicular atresia have passed through the same stages, with some differences in detail, as normally maturing egg-cells. The later stages however are different. The next step in degeneration is the breaking down of the spindles. The usual course is for the oöcytes to form the second polar spindles which then break down; but if degeneration has proceeded a little more rapidly, this fate may overtake the first polar spindles before the polar body is formed.

As stated above, in the first polar spindle as well as in the second, the achromatic fibers are all intermingled, those with chromosomes attached and those without, and do not differ in appearance or staining reaction. The fibers with no chromosomes attached to them break across at their middle and the free ends move out in the cytoplasm. As the polar ends remain attached to the poles of the spindles, the formation of "asters" is brought about. Stages are seen (Figs. 5 and 6) in which the breaking or splitting off of the fibers is taking place; some of the fibers have just broken across, in the figures, and others have already assumed a radial position, giving the appearance of "asters." The achromatic fibers with chromosomes attached next break or split off, and as their free ends move out into the cytoplasm, they draw after them the attached chromosomes (see Fig. 7). In this way more fibers are added to the "asters" and chromosomes are seen connected with the ends of some of the fibers. This splitting off of the achromatic fibers explains the fact that some spindles have radiating fibers or "asters" at their poles, while in others they are absent. The oöcytes containing spindles without radiating fibers have not advanced so far in degeneration that the spindle fibers have begun to split off. The result of this splitting off of the fibers and the consequent breaking down of the spindles is that the chromosomes are scattered in all directions in the cytoplasm at each pole of the spindle, while still connected with the poles by the spindle fibers.

The cause of the breaking down of the spindles is to be found in the degeneration of the oöcyte. That this degeneration has proceeded to quite an extent is shown by the presence in the cytoplasm of fat-granules and crystalloid bodies, and by the

fact that the cytoplasm stains much more deeply with acid stains such as eosin and orange G than does the cytoplasm of normal oöcytes. The spindle fibers share in this degeneration and show it first by breaking across and splitting off from the spindle. Rubaschkin states that as the fibers split off, the poles of the spindles approach each other and finally come to lie so close together that it is difficult or impossible to distinguish one from the other. While this account of the breaking down of the spindles agrees essentially with that of Rubaschkin for the guinea-pig, nothing resembling the approach of the poles of the spindles was observed in the mouse.

The achromatic fibers soon disappear and the chromosomes thus left free in the cytoplasm of the oöcyte begin to form nuclei. Each chromosome forms a small vesicle which has the appearance of a vacuole with the chromatin material massed at one side (Fig. 13). In some instances the chromatin is arranged in small granules around the outer part of the vesicle (Fig. 8). As this process goes on, the vesicles near enough together coalesce to form larger ones (Figs. 8, 9, 11), while those isolated in the cytoplasm remain separate. In this way a varying number of nuclei are formed, of different sizes. A nucleus formed by the combining of a number of chromosomes is larger than one formed from a single chromosome. The final number of nuclei thus formed may be from two to twelve, depending on how the chromosomes were scattered in the oöcyte. These nuclei are transformed into resting nuclei of more or less normal appearance.

The nucleo-cytoplasmic relationship, already interfered with by the degenerative changes in the egg-cell, is further disturbed by this formation of a number of small nuclei. The size-relationship, as well as the morphological, physiological, and chemical, relationship, is clearly affected. Apparently there is an effort, even in the degenerating oöcyte, to restore as far as possible this size-relationship, and this effort is expressed by a breaking up of the cytoplasm into smaller parts around the various nuclei. A part of the cytoplasm may surround several of these small nuclei when these are close together, or may enclose only one, when they are isolated. It occasionally happens that a bit of the cytoplasm may fail to contain even one of these nuclei, when

part of the egg-cell was without any nuclei as a result of an incomplete scattering of the chromosomes. The result of this breaking up of the oöcyte is that there are formed a number of small "cells," some with several nuclei, some with one, and some with none, so that the oöcyte has the appearance of a "morula." The fact that some "cells" have nuclei and others have not, is due to the uneven distribution of the chromosomes in the cytoplasm of the oöcyte when the spindle breaks down. In general, the "cells" containing large nuclei, or a group of nuclei, are larger than those with one nucleus or none. However, a definite or effective control over this fragmentation is apparently lacking.

Several authors, Newman among others, have described cells in this "morula" stage which have spindles in them, and state that these are cleavage spindles and that therefore this is a case of parthenogenetic cleavage. It is more probable, however that in such cases the cell containing the spindle is the first polar body, which, as noted above, occasionally forms a spindle, and which may in rare instances divide. In the white mouse no spindle was found in any of the cells of this "morula" stage.

In the cells of the "morula," and sometimes in the oöcyte before it has fragmented, are frequently found crystalloid bodies the nature and origin of which are unknown. Possibly they are a product of the degenerative changes in the egg-cell. Fat granules are found in the oöcytes in increasing numbers as degeneration goes on.

In a few instances the oöcyte is found to have formed two cells of nearly equal size, each containing a nucleus. Van der Stricht describes such cases in the bat and states that each cell may divide again, and each of the four cells thus formed may also divide. The formation of two such equal cells may be explained on the grounds that the scattered chromosomes were arranged in two groups and formed two nuclei; the oöcyte then broke up into two fragments of equal size. Such an egg-cell is shown in Fig. 10, with the first polar body also present; but the two nuclei are not equal in size, nor normal in appearance. In fact, one is apparently little more than a vacuole.

The fate of the "morula" may be briefly described. The zona pellucida usually persists as a thick transparent membrane

for some time after the oöcyte itself has completely degenerated and disappeared, although in a few instances it is absorbed early. Phagocytic (?) cells make their way into the oöcyte through the zona pellucida, and are probably to be regarded as follicle cells from the degenerating follicle. These cells are usually seen in the outer border of the oöcyte, or just outside it, lining the inner surface of the zona pellucida, sometimes as early in the course of degeneration as the spindle stage. There are not many of these cells in a single oöcyte, not more than eight or ten and frequently no more than three or four. They may be imbedded in the cytoplasm of the egg-cell and in the "morula" stage are frequently seen in between the separate cells. One case is illustrated (Fig. 14) showing one of these extra-ovular cells just after it has entered the oöcyte, still retaining its connection with other follicle cells outside the egg-cell by means of a protoplasmic process extending through the zona pellucida. This same cell is also shown to be connected with one or two other similar cells within the zona by other protoplasmic processes, forming a sort of syncytial net-work or mesh-work in among the fragments of the degenerating egg-cell.

The cytoplasm of these cells is usually rather scanty and sometimes they look like bare nuclei imbedded in the cytoplasm of the oöcyte (Figs. 8 and 9). They are not, however, to be confused with the nuclei of the oöcyte formed by the breaking down of the spindle, for they react differently to the stains used and have a different structure. They are finely granular and these granules are stained an intense black by iron hematoxylin and deep blue by Jenner's stain.

It may be through the action of these cells that the fragments of the oöcyte are gradually absorbed and disappear, for later on the zona pellucida is seen, shrunken and distorted, with a few of these cells in a remnant of the egg-cell. In still later stages, these cells are seen alone inside the zona, and this condition may persist for some time (Fig. 16). Eventually the zona pellucida and these cells all disappear and by this time the follicle itself has usually completely degenerated.

Thus it is seen that the oöcytes in atretic follicles in the ovary of white mice not yet sexually mature undergo a series of changes

which in the early stages at least resemble maturation. That these changes are in some way correlated with the atresia is shown by the fact that all the oöcytes exhibiting these phenomena are found in atretic follicles, and egg-cells of normal appearance are seen in the follicles not yet overtaken by atresia. The degeneration of the follicle stimulates the oöcyte to pass through a process which at first resembles maturation but which later results in a breaking up of the egg-cell into fragments, some with nuclei and some without. In the light of the evidence here presented, this process can not be considered parthenogenetic cleavage for no mitotic figures other than those of a more or less abnormal maturation were seen; and if this were true parthenogenetic cleavage it would be expected that some stages of mitosis would be observed. The absence of mitotic figures other than more or less normal polar spindles, the breaking down of these spindles, the scattering of the chromosomes, the formation of nuclei from these chromosomes, and the consequent breaking up of the egg-cell into small parts, with or without nuclei, show rather conclusively that in the white mouse, not yet sexually mature, the process is one of degenerative fragmentation.

Summary.

The spindles seen in oöcytes in follicles undergoing atresia folliculi are maturation spindles, more or less abnormal, and not cleavage spindles.

By the splitting off of the achromatic fibers and the consequent breaking down of these spindles the chromosomes are scattered through the cytoplasm of the oöcyte and form a number of nuclei.

The nucleo-cytoplasmic relationship, disturbed by the degenerative changes in follicle and egg-cell, causes the oöcyte to break up into fragments, some with one or more nuclei and some with none. These fragments are gradually absorbed, probably through the action of phagocytic cells of follicular origin, and disappear.

The process is one of degenerative fragmentation and not parthenogenetic cleavage.

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EXPLANATION OF PLATES.

All the figures are camera-lucida drawings made from the actual preparations. All the drawings except Figs. 3, 7, and 14, were made by Miss Cora J. Whitman.

PLATE I.

FIG. 1. Primary öocyte, containing a first polar spindle. The chromosomes are longer and more slender than usual in first polar spindles. The zona pelludica is seen surrounding the egg-cell (zp.). × 670.

FIG. 2. Primary oöcyte containing a first polar spindle, of the "barrel-shaped" type. The chromosomes are of the type usual for this spindle. The zona pellucida has disappeared. × 670.

FIG. 3. Secondary oöcyte, with second polar spindle and polar body which also contains a spindle more or less deranged. Some of the chromosomes of the egg spindle have been omitted from the drawing in order to show more clearly the characteristic shape of the chromosomes of the second polar spindle. × 670.

FIG. 4. Secondary oöcyte, with second polar spindle and polar body. Centrosomes are seen at each end of the spindle. × 916.

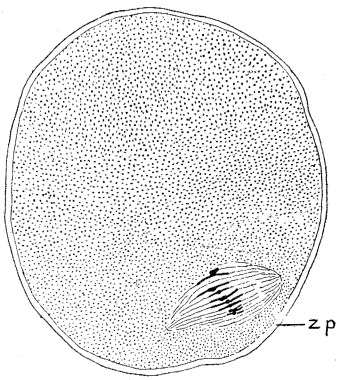

Fig 1

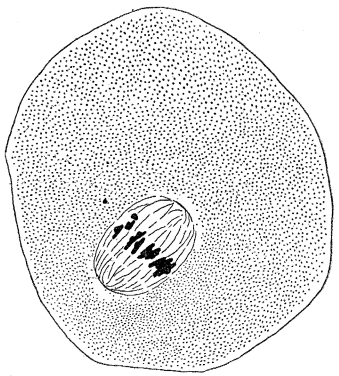

Fig 2

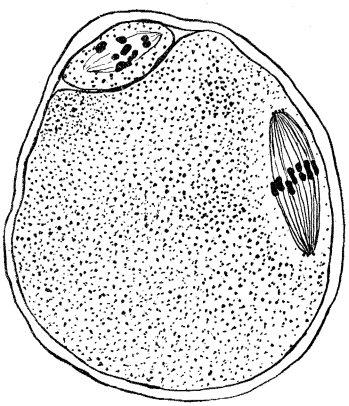

Fig 3

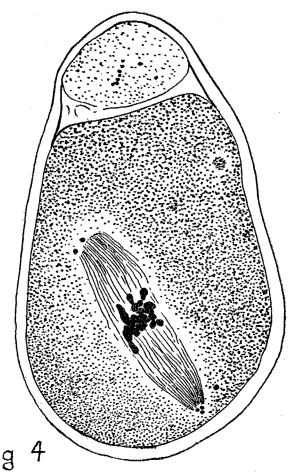

Fig 4

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PLATE II.

FIG. 5. First polar spindle alone, showing achromatic fibers splitting off, forming "asters." × 916.

FIG. 6. Second polar spindle alone, showing well-defined "asters," formed by fibers which have split off from the spindle. × 916.

FIG. 7. Primary oöcyte showing spindle seen obliquely from one end, which has broken down. The scattering of the chromosomes is partially accomplished. ×670.

FIG. 8. Egg-cell with nuclei formed from scattered chromosomes. The bodies at the end of the oöcyte (*x*) are probably cells formed by the division of the polar body. A crystalloid body (*c*) and an extra-ovular cell (*p*) are also shown. ×670.

Fig. 5

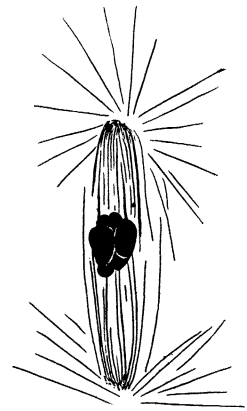

Fig. 6

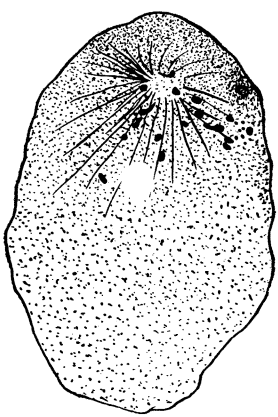

Fig. 7.

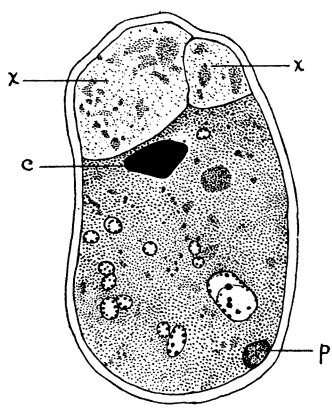

Fig. 8.

PLATE III.

FIG. 9. Egg-cell containing four nuclei; these, from their size, have been formed by the coalescence of a number of smaller vesicles. Two extra-ovular cells (*p*) are shown. × 670.

FIG. 10. Oöcyte divided into two more or less equal parts, with polar body also present (*pb*). The nuclei are not alike, one being apparently only a hollow vesicle. × 670.

FIG. 11. Egg-cell in several fragments, one of which contains five nuclei. The zona pellucida is broken in two places and extra-ovular cells are present between the fragments. × 670.

FIG. 12. "Morula" stage, some fragments with nuclei and others without. × 670.

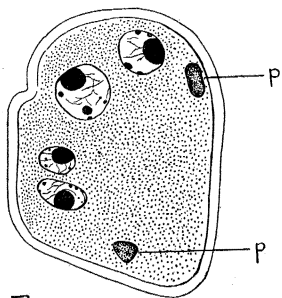

Fig 9

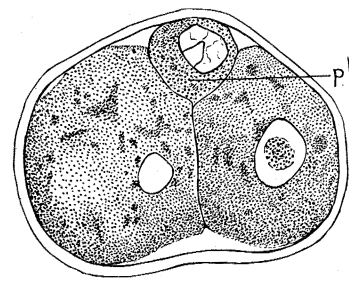

Fig 10

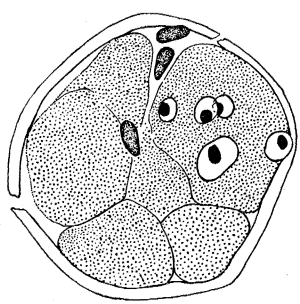

Fig 11

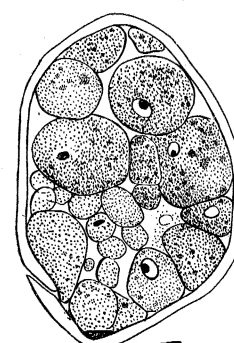

Fig 12

PLATE IV.

FIG. 13. Egg-cell showing small nuclei formed from the scattered chromosomes. The chromatin material is massed at one side of each of the vesicles. × 670.

FIG. 14. Egg-cell containing extra-ovular cells connected by protoplasmic processes. One is still connected with the follicle cells outside by a process extending through the zona pellucida. × 670.

FIGS. 15 AND 16. Final stages in degeneration. Zona pelludica with remnant of oöcyte and a few extra-ovular cells inside. × 670.

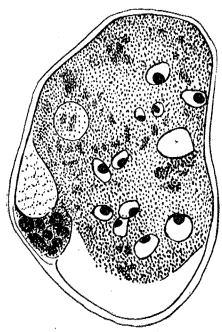

Fig. 13

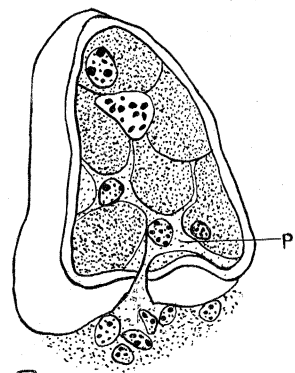

Fig 14

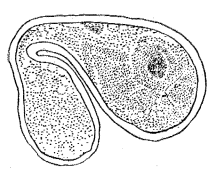

Fig 15

Fig. 16

H. M. KINGERY